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Hepatic Iron in African Americans Who Underwent Liver Biopsy

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Abstract: *Background:* Primary iron overload in African Americans has been reported predominantly from autopsy studies. *Methods:* We characterized hepatic iron phenotypes in 83 African Americans who underwent liver biopsy during the interval 1990 to 1995. We tabulated pathology report form data, iron grades in hepatocytes (0–4) and Kupffer cells (0–3) and abnormal liver histology. Increased iron was defined as hepatocyte or Kupffer iron grades ≥2, respectively. Heavy iron was defined as hepatocyte iron grade 3 or 4. Primary iron overload was defined as the presence of grade 3 or 4 hepatocellular iron in the absence of evidence of chronic alcohol effect, viral hepatitis, steatosis, unexplained inflammation, chronic erythrocyte transfusion or chronic ingestion of iron supplements. *Results:* There were 37 men and 46 women (mean age: 53 ± 15 [SD] years). We observed heavy ethanol consumption, 12.0%; viral hepatitis, 26.5%; steatosis without heavy ethanol consumption, 43.4%; inflammation, 45.6%; fibrosis, 26.2% and bridging fibrosis/cirrhosis, 29.4%. Logistic regression on bridging fibrosis/cirrhosis revealed positive associations with heavy ethanol consumption ($P = 0.0410$) and viral hepatitis ($P = 0.0044$). The 22 patients (26.5%) with increased iron had greater mean age, proportion of men and heavy ethanol consumption. Five patients had heavy iron staining, among whom were 3 women (mean age: 54 years) with primary iron overload. Two of the 3 women had cirrhosis and diabetes mellitus. *Conclusions:* Among 83 adult African Americans who underwent liver biopsy, 3.6% had hepatic iron phenotypes consistent with primary iron overload.

Key Indexing Terms: African Americans; Hemochromatosis; Hepatocyte; Iron overload; Liver. **[Am J Med Sci 2015;349(1):50–55.]**

BACKGROUND

Primary iron overload in African Americans is a heterogeneous group of disorders. DNA analysis as an aid to diagnosis of some primary iron overload disorders became possible after the discovery of the *HFE* gene (chromosome 6p21.3) in 1996.[1] In whites, for example, *HFE* genotypes, especially p.C282Y homozygosity, account for approximately 90% of hemochromatosis phenotypes.[1,2] In contrast, hemochromatosis-associated *HFE* genotypes are uncommon in African Americans with high-iron phenotypes.[3,4] Deleterious mutations in non-*HFE* iron-related genes have been described in few African Americans with primary iron overload unassociated with anemia.[5–7] Population screening to identify primary iron overload in African Americans has been performed using an elevated transferrin saturation or serum ferritin criterion like that used in screening whites.[4,8] The prevalence of possible or confirmed cases was very low[4,8] in part because mean transferrin saturation is lower and mean serum ferritin is higher in African Americans than whites.[4] Thus, the pretreatment diagnosis of primary iron overload in African Americans depends predominantly on demonstration of high-iron phenotypes detectable in the liver.

Grading stainable iron in hepatocytes and Kupffer cells is a basic hepatic iron phenotyping method. There is a significant positive correlation of hepatocyte iron grade with hepatic iron concentration (HIC) measured using atomic absorption spectrometry.[9] The hepatic iron index (HII) is HIC adjusted by age (μmol Fe/g dry weight/y).[10,11] HII ≥1.9 confirms the diagnosis of iron overload and has been used as a diagnostic criterion of primary iron overload in African Americans.[12,13]

Most high-iron phenotypes in African Americans without anemia have been communicated as case reports,[3,14,15] small case series[12,13] or autopsy studies.[13,16–18] We performed a retrospective study of consecutive diagnostic liver biopsy specimens of African American adults in a large suburban medical center in central Alabama during the interval 1990 to 1994, a period during which DNA-based diagnosis of primary iron overload disorders was not available. We identified and characterized hepatic iron phenotypes using hepatocyte and Kupffer cell iron grades. We discuss our results in the context of the overall prevalence of high-iron phenotypes in African Americans and acquired factors that may contribute to their development.

METHODS

Selection of Study Subjects

The performance of this study was approved by the Institutional Review Board of Brookwood Medical Center. We performed a computerized and manual search of the database of the Medical Center's surgical pathology department to identify all pathology report forms of liver specimens obtained by percutaneous or intraoperative biopsy from adults (age ≥18 years) during the 5-year study interval 1990 to 1994. Thereafter, we selected patients whose pathology report forms identified them as either African American or black. One hundred African Americans underwent liver biopsy during the study interval. We retrieved their liver biopsy slides and tabulated the succinct demographic data, clinical history and pathologist's interpretation of the biopsies. Using hospital or clinic charts or blood bank records was not part of the present study.

Performance of Liver Biopsies

Liver specimens were obtained by biopsy as part of routine medical care. No biopsy specimen was obtained as a sequel to population or family screening to detect iron overload

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phenotypes. Radiologists or gastroenterologists obtained specimens using percutaneous technique, 18-gauge needles and ultrasound or CT guidance. Surgeons obtained specimens during open cholecystectomy or other intraperitoneal operations.

Selection of Evaluable Liver Biopsy Specimens

We excluded biopsy specimens less than 10 mm long. Thus, the specimen of 1 woman was inevaluable because there was insufficient liver for interpretation. We excluded 15 other cases because the biopsies were performed to confirm the diagnosis of malignancy and not to evaluate parenchymal liver disease. Furthermore, none of these biopsy specimens had sufficient liver parenchyma for analysis. The biopsy specimen of 1 man was excluded because his pathology report indicated that he had transfusion iron overload consequent to treatment for acute leukemia. Altogether, there were 83 specimens evaluable for the present analyses.

Histology Technique

Liver specimens were fixed in 10% neutral buffered formalin. Triplicate sections of paraffin-embedded liver were routinely prepared. One section was stained with hematoxylin and eosin, another with Perls' acid ferrocyanide technique to demonstrate nonheme ferric iron and a third with Masson's trichrome technique to assess collagen fibrosis. In some cases, reticulin stains were also used to assess fibrosis. Other special stains were used in some cases, as appropriate. Appropriate positive and negative control specimens were prepared and reviewed with each staining batch.

Liver Morphology

Interpretation of liver histology reported herein represents consensus opinions of the surgical pathologist and at least 2 of the authors. Steatosis unassociated with excessive ethanol consumption, inflammation and bridging fibrosis/cirrhosis was assessed as described elsewhere.[19] The abnormality was graded as absent or present. The presence or absence of fibrosis, bridging fibrosis or hepatic cirrhosis was determined using Masson's trichrome-stained specimens with or without reticulin stains as described previously.[19] Fibrosis without bridging or cirrhosis was graded as either present or absent. We defined that bridging fibrosis and cirrhosis were equivalent; their presence or absence was analyzed as a dichotomous variable. The presence of abnormalities characteristic of viral hepatitis was based on a combination of information obtained from pathology request forms and histologic features of the biopsy specimens.

Iron Grading

All slides were reviewed by a surgical pathologist and at least 2 of the authors. The iron grades in each case represent consensus opinions. Hepatocellular iron was graded according to these criteria: grade 0—no visible iron; grade 1—iron visible in very few hepatocytes; grade 2—iron visible in 5% to 10% of hepatocytes; grade 3—iron visible in ≥40% of hepatocytes and grade 4—abundant iron visible in most hepatocytes.[12] Kupffer cell iron was graded according to these criteria: grade 0—no visible iron in Kupffer cells; grade 1—iron visible in ≥one-third of Kupffer cells; grade 2—iron visible in one-third to ≤two-thirds of Kupffer cells and grade 3—abundant iron visible in more than two-thirds of Kupffer cells.[12] Hepatocyte or Kupffer cell iron of grade 0 or 1 was defined as normal.

Increased iron was defined as hepatocyte and/or Kupffer cell iron grade ≥2.[12] Heavy iron staining was defined as hepatocyte iron grade 3 or 4, regardless of Kupffer cell iron grade. Primary iron overload was defined as the presence of grade 3 or 4 hepatocellular iron in the absence of evidence of chronic alcohol effect, viral hepatitis, steatosis, unexplained inflammation, chronic erythrocyte transfusion or chronic ingestion of iron supplements.

Hepatic Iron Concentration

The reference range for HIC measured by atomic absorption spectrometry is 200 to 2400 μg Fe/g dry weight (3.6–43.0 μmol Fe/g dry weight). In only 1 patient was the HIC measurement requested by the interpreting pathologist and displayed in the report form. Measuring HIC on the biopsy specimens as an addendum to the data on pathology report forms was beyond the scope of the present study.

Other Conditions

These were tabulated from information on the pathology request forms and included the following: reports of heavy ethanol consumption (usually not otherwise specified), positive serologic reactions or quantitative or qualitative RNA assessments for viral hepatitis B or C, elevated blood iron measures (defined as serum iron concentration, transferrin saturation or serum ferritin concentration), history of erythrocyte transfusion, consumption of iron supplements, numbers of pregnancies and histories of thalassemia and other heritable or acquired types of anemia. Some conditions we tabulated were taken from the pathologist's histologic interpretations of the liver biopsy specimens.

Statistical Analyses

The present data set consisted of observations in 83 consecutive adult African Americans whose liver biopsy specimens were evaluable. Analyses were performed with a computer spreadsheet (Excel 2000; Microsoft Corp, Redmond, WA) and a statistical program (GB-Stat version 10.0, 2003; Dynamic Microsystems, Inc, Silver Spring, MD). Descriptive data are displayed as enumerations, percentages and mean ± 1 SD. Frequency values were compared using Pearson's χ^2 analysis or Fisher's exact test, as appropriate. Mean values were compared using 1-tailed student *t*-test. Some data were analyzed using Pearson's correlation coefficient. We performed logistic regressions on increased stainable iron and cirrhosis. All independent variables except age (continuous variable) were dichotomous. Values of $P < 0.05$ were defined as significant.

RESULTS

General Characteristics of 83 Patients

There were 37 men (44.6%) and 46 women (55.4%) (Table 1). Liver biopsy was performed using percutaneous technique in 64 patients (77.1%) and intraoperative technique in the remaining 19 patients (22.9%). The mean ages of men and women were similar (52 ± 16 years and 54 ± 14 years, respectively; $P = 0.5201$). There were reports of heavy ethanol consumption in 10 patients (12.0%), viral hepatitis in 18 patients (26.5%), steatosis unassociated with a report of heavy ethanol consumption in 36 patients (43.4%), inflammation in 31 patients (45.6%), fibrosis in 16 patients (26.2%) and bridging fibrosis/cirrhosis in 20 patients (29.4%) (Table 1). Elevated blood iron measures were reported in 4 patients (4.8%). No patient had a history of anemia treated with erythrocyte transfusion.

Comparisons of Patients With and Without Increased Stainable Liver Iron

Twenty-two patients (26.5%) had increased stainable iron (Table 1). Their mean age, the proportion of men and the reports of heavy ethanol consumption were greater than in patients

TABLE 1. Characteristics of 83 African Americans who underwent liver biopsy

Characteristics	Increased stainable iron (n = 22)	No increased stainable iron (n = 61)	*P*
Mean age ± 1 SD, y	58 ± 16	51 ± 16	0.0304
Men, % (n)	68.2 (15)	36.1 (22)	0.0094
Heavy ethanol, % (n)	31.8 (7)	4.9 (3)	0.0028
Viral hepatitis, % (n)[a]	22.7 (5)	21.3 (13)	0.5540
Steatosis, % (n)[b]	22.7 (5)	50.8 (31)	0.0226
Inflammation, % (n)[b]	31.8 (7)	39.3 (24)	0.5316
Fibrosis, % (n)[b]	13.6 (3)	21.3 (13)	0.3301
Cirrhosis, % (n)[b]	31.8 (7)	21.3 (13)	0.3232
Elevated blood iron measures, n (%)[c]	9.1 (2)	3.3 (2)	0.2695
Thalassemia, % (n)	9.1 (2)[d]	0.0 (0)	0.0679
Percutaneous biopsy technique, % (n)	90.9 (20)	0.0 (0)	0.0679

[a] Defined as hepatitis C by clinical history or hepatic histology on pathology reports except hepatitis B in 1 patient.
[b] Steatosis, inflammation, and fibrosis were assessed as described in detail elsewhere.19 Cirrhosis includes bridging fibrosis, by definition. These abnormalities were graded as present or absent.
[c] Reports of increased serum iron, transferrin saturation or serum ferritin levels.
[d] A 63-year-old man had reports of alpha-thalassemia trait, heavy ethanol consumption, cirrhosis, liver iron concentration 3596 μg/g dry weight and HII 1.0. A 43 year-old woman had reports of beta-thalassemia trait, cirrhosis, diabetes mellitus, and hypopituitarism.

without increased stainable iron. The proportion of patients with steatosis unassociated with increased ethanol consumption was lower among those with increased stainable iron than among those without increased stainable iron (Table 1). The proportions of patients with and without increased stainable iron who had reports of increased blood iron measures or who underwent percutaneous biopsy did not differ significantly (Table 1).

Characteristics of 22 Patients With Increased Stainable Iron

There were 15 men (68.2%) and 7 women (31.8%). The proportions of men and women with increased stainable iron in hepatocytes or Kupffer cells did not differ significantly. Four patients had increased hepatocyte staining only (18.2%) and 6 patients (27.3%) had increased Kupffer cell staining only. Twelve patients (54.5%) had a mixed pattern of increased hepatocyte and Kupffer cell iron staining. Elevated blood iron measures were reported in 2 patients (9.1%). Analysis of hepatocyte and Kupffer cell iron grades revealed a Pearson correlation coefficient of 0.2260 ($P = 0.3120$).

Characteristics of 5 Patients With Heavy Iron Staining

Heavy iron staining was observed in 1 of 37 men (2.7%) and 4 of 46 women (8.7%) ($P = 0.4471$). The mean age of these 5 probands was 60 years (Table 2). Four of these patients underwent percutaneous biopsy. None had a prebiopsy report of elevated blood iron measures. Each had a mixed pattern of heavy staining in both hepatocytes and Kupffer cells (Table 2). None had reports of heavy ethanol consumption or histologic evidence of hepatic steatosis (Table 2).

A 73-year-old woman had viral hepatitis without report of another abnormality. A 66-year-old man had cirrhosis and hepatic inflammation without report of another abnormality.

Three women had primary iron overload as defined herein. Their mean age was 54 years. Two of the 3 women had cirrhosis and diabetes mellitus (Table 2).

Logistic Regressions on Increased Stainable Iron and Cirrhosis

Regression on increased stainable iron was performed using age, sex, heavy ethanol consumption, viral hepatitis, steatosis, inflammation, fibrosis, bridging fibrosis/cirrhosis and thalassemia observations from all 83 evaluable patients as independent variables. There was no significant association of any of these independent variables with increased stainable iron. We performed a regression on cirrhosis using age, sex, increased stainable iron, heavy ethanol consumption, viral hepatitis, steatosis, inflammation, fibrosis, bridging fibrosis/cirrhosis and thalassemia as independent variables. Bridging fibrosis/cirrhosis was positively associated with heavy ethanol consumption ($P = 0.0410$) and viral hepatitis ($P = 0.0044$).

TABLE 2. Five African Americans with heavy liver iron staining[a]

Age (y), sex	Hepatocyte iron grade	Kupffer cell iron grade	Viral hepatitis	Inflammation	Cirrhosis[b]
43 F[c]	3	2	0	0	+
44 F	3	3	0	0	0
66 M	3	2	0	+	+
73 F	3	2	+	0	0
76 F[d]	4	3	0	0	+

[a] Heavy iron staining was defined as hepatocyte iron grade of 3 or 4, regardless of Kupffer cell iron grade. None of these patients had heavy ethanol consumption or hepatic steatosis or fibrosis.
[b] Includes bridging fibrosis, by definition.
[c] This woman had reports of beta-thalassemia trait, diabetes mellitus and hypopituitarism.
[d] This woman had diabetes mellitus.

DISCUSSION

We observed primary iron overload in 3 of the present 83 African American adults (3.6%) who underwent liver biopsy. All were women (mean age: 54 years). Two of the 3 women had cirrhosis and diabetes mellitus. None of these 3 women had chronic ethanol consumption, evidence of viral hepatitis, steatosis, unexplained hepatic inflammation or reports of previous erythrocyte transfusion or consumption of iron supplements.

We chose the study interval 1990 to 1994 because iron overload diagnosis at that time depended exclusively on iron phenotyping. In a 1996 study, 4 of 326 African American adults (1.2%) who died in hospital and underwent autopsy had HII ≥1.9 (1.9–5.6), after adjustment for erythrocyte transfusions.[13] There were 2 men and 2 women (mean age: 56 years). Wurapa et al[13] concluded that these 4 patients had primary iron overload. Among 4,573 African American adults in a large university hospital autopsy series, 7 (0.15%) had severe multiorgan iron overload unassociated with erythrocyte transfusion.[17] Six of the 7 patients had clinical histories and pathologic findings typical of primary African American iron overload, and the other patient had a clinical and pathologic picture consistent with early age-of-onset (juvenile) hemochromatosis.[17]

There is a significant positive correlation of hepatocyte iron grades with HIC in subjects in whom the clinical manifestations and histologic distribution of hepatic iron suggest hemochromatosis, that is, predominance of hepatocyte iron and no apparent explanation for iron overload.[20] HII was conceived and validated as an aid to the diagnosis of hemochromatosis homozygosity in whites[10,11] before mutation analysis for pathogenic *HFE* alleles became possible in 1996.[1] In whites, HII ≥1.9 is sufficiently high to confirm the diagnosis of hemochromatosis homozygosity and exclude lesser degrees of HIC for age, that is, typically associated with heavy ethanol consumption or hemochromatosis heterozygosity.[10,11] HII has also been used as a conservative surrogate diagnostic criterion for primary iron overload in African Americans[3,12,13,16] and other types of nonhemochromatosis hepatic or systemic iron overload.[21,22] Measuring HII was beyond the scope of the present work.

Elevated blood iron measures were not mentioned on the pathology report forms of any of the 3 present women who had primary iron overload as defined herein. Clinicians treating most of the present patients may not have considered primary iron overload as a possible cause of liver disease because few cases of primary iron overload in African Americans were reported before 1995.[14,15] In a survey of 2,563 physicians in the United States published in 2002, only 32% reported correct answers about diagnosis of hemochromatosis,[23] although hemochromatosis in whites was well described in the U.S. literature before 1990.[24–27] It is also possible that assessments of blood iron measures performed before liver biopsy in some of the patients were not reported on their pathology request forms.

The prevalence of increased liver iron staining was greater in men than women in the present study. In 341 African Americans who underwent coroner's autopsy, there was a significant correlation of male sex with hepatocyte iron grade.[17] Higher testosterone levels in men could downregulate hepcidin, resulting in greater iron absorption and iron stores in men than in women.[28] The iron losses of menstruation, pregnancy and lactation could also account for lower iron stores in women than men, on the average. Nonetheless, documentation of voluntary blood donation, reports of medications or illnesses that cause blood loss or that would impair iron absorption and reports of iron supplements, numbers of pregnancies and access to blood bank records of erythrocyte transfusion were not available for the present analyses.

The prevalence of heavy ethanol consumption reports was greater in the present patients with increased liver iron staining, consistent with observations in cohorts of African Americans with nontransfusion iron overload.[12,13] Logistic regressions on cirrhosis revealed positive associations with reports of heavy ethanol consumption. Heavy ethanol consumption downregulates hepcidin and thus could contribute to increased iron absorption.[29] Chronic alcoholism is a common cause of cirrhosis in North America. In persons with cirrhosis, regardless of cause, serum hepcidin levels are lower. This is presumed to reflect dysregulation of iron sensing and decreased hepcidin production by cirrhotic livers.[30]

More than one-fifth of the present patients had evidence of chronic viral hepatitis, mostly hepatitis C. There may have been a selection bias to perform liver biopsy in patients who had proven or suspected chronic viral hepatitis. A greater proportion of African Americans than persons of other races respond to chronic hepatitis C infection with an increase in iron stores, after adjustment for age, alcohol intake, gender, menopausal status, education, body mass index and poverty index.[31] Regardless, the proportions of the present patients with viral hepatitis did not differ significantly between those with and those without increased liver iron staining. Logistic regressions on cirrhosis revealed positive associations with viral hepatitis in the present cohort. Chronic viral hepatitis is a common cause of cirrhosis in North America.

Hepatic steatosis unassociated with reports of heavy ethanol consumption was present in 43% of the present 83 patients. Our prevalence estimate may have been high because we did not stratify patients by severity of steatosis or may have been low because some patients with steatosis had heavy ethanol consumption unreported on pathology request forms. In another study of liver biopsies, 18% of 301 African Americans without histories of increased ethanol consumption had hepatic steatosis grade 1, 2, 3 or 4.[32]

Hepatic steatosis or increased body mass index may promote iron deposition in the liver.[33,34] Hepatic iron sensing is abnormal in nonalcoholic fatty liver disease but hepcidin production is increased.[35,36] Increased hepatic iron retention may be due to decreased ferroportin expression in hepatocytes and consequent decreased iron export.[35] In addition, increased hepatic iron may act in concert with steatosis to promote liver injury.[33,34,37] In 30 African American adults discovered to have heavy hepatic iron staining at coroner's autopsy, there were significant positive correlations of steatosis with inflammation and of inflammation with bridging fibrosis/cirrhosis.[17] In the present study, steatosis was not associated with increased iron staining in univariable analyses or with cirrhosis in regression analyses. Steatosis was not observed in any of the 3 present patients with HII ≥1.9. Similarly, nonalcoholic steatosis demonstrated by liver biopsy or ultrasonography/computerized tomography scan criteria in white hemochromatosis probands with *HFE* C282Y homozygosity was not a predictor of iron overload measured by quantitative phlebotomy.[38]

Erythrocyte transfusion is an unlikely cause of iron overload in the present 83 patients. Among the 100 patients whose pathology reports we initially reviewed, 1 man was excluded because he had a report of transfusion iron overload associated with treatment of acute myelogenous leukemia. It was reported that 2 of the evaluable 83 patients had minor types of thalassemia. Erythrocyte transfusion is not routinely administered in such patients because anemia, if any, is mild. Although erythropoiesis is mildly ineffective in minor types of thalassemia, iron absorption is not increased to the extent that iron overload occurs[39] except in some patients who have inherited other iron overload-related alleles.[2,40]

There are other uncertainties in the present results. The criteria used by physicians and surgeons to advise their patients to undergo liver biopsy were probably variable and may have led to selection bias in the present cohort. The number of African Americans believed to have nontransfusion iron overload at our medical center during the interval 1990 to 1994 but who did not undergo liver biopsy is unknown. Some African Americans with primary iron overload reported previously had malignancy.[12,13] We excluded 15 patients from the present analyses because their liver biopsies contained cancer, but there was insufficient liver parenchyma for evaluation of iron staining and liver histology. It is unknown if any of these patients had increased liver iron. Physicians practicing in this geographic area today may be more familiar with iron overload in African Americans than they were in the interval 1990 to 1994, although this is unproven. Some African Americans previously reported to have had iron overload by quantitative phlebotomy criteria had normal HIC and HII <1.9.[12] This implies that other patients in the present cohort may have primary iron overload that was undetected by the methods used in this study. HIC measurements, if performed in all of the present cases, would have provided more information about HII in African Americans. Nonetheless, it is unlikely that having such measurements would have changed our estimation of the proportion of patients who had heavy iron staining defined herein as primary iron overload. The occurrence of common *HFE* missense mutations or deleterious mutations in non-*HFE* genes and family history of disease in the present patients is unknown.

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